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MAJOR ARTICLE

Host- and Microbe-Related Risk Factors for and Pathophysiology of Fatal *Rickettsia conorii* Infection in Portuguese Patients

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Background. The pathophysiologic mechanisms that determine the severity of Mediterranean spotted fever (MSF) and the host-related and microbe-related risk factors for a fatal outcome are incompletely understood.

Methods. This prospective study used univariate and multivariate analyses to determine the risk factors for a fatal outcome for 140 patients with *Rickettsia conorii* infection admitted to 13 Portuguese hospitals during 1994–2006 with documented identification of the rickettsial strain causing their infection.

Results. A total of 71 patients (51%) were infected with the Malish strain of *Rickettsia conorii,* and 69 (49%) were infected with the Israeli spotted fever (ISF) strain. Patients were admitted to the intensive care unit (40 [29%]), hospitalized as routine inpatients (95[67%]), or managed as outpatients (5[4%]). Death occurred in 29 adults (21%). A fatal outcome was significantly more likely for patients infected with the ISF strain, and alcoholism was a risk factor. The pathophysiology of a fatal outcome involved significantly greater incidence of petechial rash, gastrointestinal symptoms, obtundation and/or confusion, dehydration, tachypnea, hepatomegaly, leukocytosis, coagulopathy, azotemia, hyperbilirubinemia, and elevated levels of hepatic enzymes and creatine kinase. Some, but not all, of these findings were observed more often in ISF strain–infected patients.

Conclusions. Although fatalities and similar clinical manifestations occurred among both groups of patients, the ISF strain was more virulent than the Malish strain. Multivariate analysis revealed that acute renal failure and hyperbilirubinemia were most strongly associated with a fatal outcome.

Mediterranean spotted fever (MSF), Israeli spotted fever (ISF), Indian tick typhus, and Astrakhan spotted fever are caused by distinct strains of *Rickettsia conorii.* These strains exhibit minor antigenic and genotypic differences and have been hypothesized to cause distinctive clinical signs, as well as disease of differing severity [1, 2].

In Portugal, MSF is caused by *R. conorii* Malish and ISF strains [3]. The incidence of MSF was 8.4 infections per 10,000 inhabitants during 1989–2005, a high rate compared with other countries in the Mediterranean basin in which MSF is endemic. In the past decade, an increasing number of cases of the malignant form of MSF have been described in Portuguese patients [4, 5]. Moreover, based on confirmed diagnoses in the Portuguese hospital database (Administração Central do Sistema de Saúde, Portuguese Ministry of Health), the number of admissions for MSF increased from 176 patients in 1994 to 446 patients in 2004 [6]. The case fatality rate during the same period was 3%–7% among hospitalized patients. In 1997, the case fatality rate was very high in 2 Portuguese hospitals, and in the Beja district, the rate reached 32% [4].

A study conducted in Beja Hospital by Sousa et al. [4] from 1994 through 1998 to identify the risk factors associated with a fatal outcome indicated that delay in antibiotic treatment of rickettsial infection was not the ex-

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Table 1. Age and treatment intervals for patients, according to strain of *Rickettsia conorii*.

Type of infection, variable	Infecting strain of *R. conorii*		*P*
	Malish	ISF	
Nonfatal			
Age, median, years	63	57	.326
Treatment interval			
1st symptom to 1st visit, median, days	4	3	.189
1st visit to appropriate antimicrobial therapy, median, days	0	0	.241
1st symptom to specific therapy, median, days	5	4	**.028**
Length of stay, median, days	7	6	.081
Fatal			
Age, median, years	66	57.5	.114
Treatment interval			
1st symptom to 1st visit, median, days	4	3	.618
1st visit to specific therapy, median, days	0	.5	.371
1st symptom to appropriate antimicrobial therapy, median, days	4.5	4	.899
Length of stay, median, days	2	1	.203

NOTE. Bold type indicates significant *P* values. ISF, Israel spotted fever.

planation; instead, the risk of death was related to comorbidities such as diabetes mellitus. It was suspected that the fatal outcome might have been related to the ISF strain, which was isolated for the first time in Portugal in 1997 from patients with fatal infections [7]. The hypothesis was that the ISF strain might cause different clinical manifestations than those caused by the Malish strain, which could lead to a late clinical diagnosis and consequently, to a delay in treatment, thus resulting in a higher number of severe cases. In 2005, Sousa et al. evaluated the 3 main signs of infection—fever, rash, eschar—and severity of disease in patients infected with ISF and Malish strains [3]. They observed no statistically significant differences in the occurrence of signs, in particular, the presence or absence of an eschar, in 94 Portuguese patients infected with either the Malish or the ISF strains. In Israel, eschars caused by infection with the ISF strain have been described in only 4% of cases [8–10]. Because our previous studies were not exhaustive, we conducted a larger study to determine the risk factors for death in Portuguese patients who had a diagnosis of MSF confirmed by identification of the strain causing the infection.

METHODS

Data Collection and Definitions

Patients were admitted to Portuguese hospitals with a clinical diagnosis of MSF confirmed by isolation of *Rickettsia* species from blood or detection of rickettsial DNA in skin biopsy sample by polymerase chain reaction (PCR) at the Portuguese National Institute of Health during 1994–2006. Additionally, serum samples were tested for the presence of IgM and IgG antibodies against *R. conorii.* Epidemiological, clinical, and laboratory data were recorded at admission. The onset of symptoms was defined as the first day on which any of the following symptoms were observed: fever, chills, rash, headache, or gastrointestinal symptoms, such as nausea, vomiting, and/or diarrhea. Alcoholism was defined as ingestion of 120 g ethanol per day, as well as on the basis of reports from family members and/or a family physician about the patient's habits with respect to alcohol. Respiratory insufficiency was defined as failure of the exchange of oxygen and carbon dioxide, $pO_2 < 60$ mm Hg or $pCO_2 > 55$ mm Hg. Congestive heart failure was defined as class III or above on the New York Heart Association Functional Classification for Congestive Heart Failure. Appropriate antibiotic treatment was defined as therapy that included least 1 antibiotic to which *R. conorii* is susceptible. Severity of disease was scored on the basis of patient admission to the intensive care unit (ICU), Acute Physiology and Chronic Health Evaluation II score (data not shown), or death.

Laboratory Confirmation of MSF

Isolation of* Rickettsia *species from blood. A heparinized blood sample was collected from patients with a presumptive clinical diagnosis of MSF at hospital admission. Plasma was tested for rickettsial antibodies by immunofluorescence assay. Blood culture was performed to isolate *Rickettsia,* and the strain causing the infection was identified, as described elsewhere [11].

DNA detection in skin biopsies. At admission, a punch biopsy was performed to obtain a 5-mm skin sample from the eschar, if present, or from a rash lesion. Informed consent was obtained from all subjects, and experiments were performed with approval of the ethical committees of the hospitals and the Portuguese National Institute of Health. DNA extraction, PCR,

Table 2. Comparative analysis of epidemiologic, clinical, and laboratory findings for patients infected by *Rickettsia conorii,* according to infecting strain.

Finding, by type	Total[a]	Patients infected with each strain of *R. conorii,* no. (%)[b]		*P*
		Malish	ISF	
Epidemiologic finding				
Animal contact	88	44 (94)	36 (88)	.465
Tick bite	66	20 (63)	11 (32)	**.014**
Clinical finding				
Fever	137	64 (94)	65 (94)	.99
Eschar	123	38 (60)	23 (38)	**.015**
Rash				
Any	137	65 (96)	63 (91)	.463
Maculopapular	120	61 (94)	59 (94)	.99
Petechial	8	4 (6)	4 (6)	.99
Asthenia	80	39 (95)	35 (90)	.426
Headache	77	32 (78)	28 (78)	.977
Myalgia	85	38 (84)	34 (85)	.943
Arthralgia	69	22 (63)	16 (47)	.187
Abdominal pain	70	7 (19)	12 (36)	.101
Anorexia	65	27 (73)	19 (68)	.653
Nausea	75	14 (35)	22 (63)	**.016**
Vomiting	84	13 (30)	23 (56)	**.017**
Diarrhea	79	11 (28)	17 (44)	.135
Prostration	81	27 (64)	26 (67)	.822
Obtundation and/or confusion	100	19 (38)	15 (30)	.398
Dehydration	72	14 (39)	17 (47)	.475
Tachypnea	63	10 (38)	18 (49)	.452
Chest pain	53	3 (14)	6 (19)	.99
Hepatomegaly	60	9 (29)	9 (31)	.866
Splenomegaly	57	2 (7)	3 (11)	.660
Comorbidity				
Alcoholism	103	9 (18)	9 (17)	.892
Tobacco use	87	6 (15)	7 (15)	.939
Diabetes	107	5 (10)	4 (7)	.737
Cardiac failure	105	8 (16)	6 (11)	.443
Respiratory failure	85	2 (5)	4 (9)	.677
Chronic renal failure	85	3 (7)	0	.116
Hypertension	89	10 (22)	7 (16)	.449
Laboratory finding[c]				
Anemia, hemoglobin level <11 g/dL	118	13 (22)	8 (13)	.197
Leukopenia, <4400 leukocytes/μL	121	6 (10)	10 (16)	.571
Leukocytosis, >11,300 leukocytes/μL	121	12 (20)	10 (16)	.571
Thrombocytopenia[d]	122	45 (76)	55 (87)	.113
Prothrombin time >13 s	84	19 (48)	24 (55)	.544
Partial thromboplastin time >35 s	95	17 (35)	22 (47)	.598
Glucose level >110 mg/dL	101	33 (66)	29 (57)	.346
Urea level[d]	112	29 (56)	32 (53)	.796
Creatinine level >1.2 mg/dL	120	36 (60)	32 (53)	.461
Sodium level <145 mmol/L	120	30 (52)	26 (42)	.283
Potassium level <3.5 mmol/L	116	10 (18)	7 (11)	.308
Total bilirubin level >1.2 mg/dL	109	14 (26)	25 (45)	**.047**
Alanine transaminase level[d]	120	37 (64)	45 (73)	.301

(continued)

Table 2. ***(Continued)***

Finding, by type	Total[a]	Patients infected with each strain of *R. conorii*, no. (%)[b]		*P*
		Malish	ISF	
Aspartate transaminase level[d]	112	42 (79)	53 (90)	.119
γ-Glutamyl transferase level[d]	77	17 (50)	32 (74)	**.027**
Alkaline phosphatase level[d]	88	10 (26)	24 (49)	**.026**
Creatine kinase level[d]	75	19 (51)	13 (34)	.133
C-reactive protein level >0.5 mg/dL	66	34 (97)	31 (100)	.343

NOTE. Bold type indicates significant *P* values. ISF, Israeli spotted fever.

[a] All patients with available data.

[b] The percentage is based on the total number of patients infected by each strain.

[c] Children were excluded from analyses of laboratory data.

[d] For these laboratory values, the abnormal values observed were considered outside the normal range limits (either below or above those limits) established by each different hospital laboratory.

and strain characterization were performed, as described elsewhere [3].

Serologic analysis. The diagnosis of *R. conorii* infection was confirmed when serum samples obtained from patients with acute infection contained IgM antibodies at a titer of ⩾32 and/or IgG titer of ⩾128 or there was a 4-fold increase in titer between serum samples from patients with acute infection and samples from convalescent patients [12, 13]. The cutoff for a positive result for *R. conorii* infection was established by the Portguese National Institute of Health on the basis of previous studies in the Portuguese population and previous studies of Portugal as a country where MSF is endemic. Serum samples from convalescent patients were collected at least 15 days after samples obtained during acute infection.

Statistical analysis. Exploratory data analysis was performed by using tables of frequencies and central tendency and dispersion measures for continuous variables. Differences were evaluated by use of Fisher's exact test or Pearson's χ^2 test for categorical variables and by use of the Mann-Whitney test when continuous variables did not show a Gaussian distribution. Differences were considered to be significant at $P \leqslant .05$. The association between death and each factor was measured by an odds ratio (OR) obtained with univariate logistic regression models. The multiple logistic regression model was built by stepwise selection, using the criteria of $P < .05$ for selection variables and $P > .10$ for backward elimination. The goodness-of-fit of the final model was evaluated by means of Pearson's χ^2 test and the Hosmer-Lemeshow test. Data analysis was performed with Stata (version 9.0 software; Stata).

RESULTS

From January 1994 through July 2006, a clinical diagnosis of MSF was confirmed in 140 patients from 13 hospitals. Most of the patients were from 5 hospitals from southern Portugal: 23 from Hospital Garcia de Orta in Almada, 39 from Centro Hospitalar do Baixo Alentejo in Beja, 32 from Hospital do Espiríto Santo in Évora, 17 from Hospital Distrital de Faro, and 17 from Centro Hospital de Setúbal. The remaining 12 patients were from 8 other hospitals. Most of the cases occurred during summer; 113 cases (81%) occurred during the period from July to September.

MSF due to infection with the Malish or ISF strains of *R. conorii* was diagnosed in 65 males (46%) and 75 females (54%). The median age was 60.5 years. Six of the patients were not adults (i.e., <18 years old): 5 were <6 years old, and 1 was 12 years old. Forty patients (29%) were admitted to an ICU, 95 (67%) were admitted to a ward, and 5 (4%) were treated as outpatients. Death due to infection with Malish or ISF strains of *R. conorii* occurred in 29 adults (21%; 15 men and 14 women).

MSF was confirmed in 93 patients by isolation of *Rickettsia* species from blood and in 47 patients by detection of *R. conorii* DNA by PCR. Additionally, serologic analysis was performed on 138 serum samples obtained from patients with acute infection and 53 serum samples obtained from convalescent patients. Of the 138 serum samples from patients with acute infection, only 4 patients (3%) had a significant level of IgM and/or IgG antibodies against *R. conorii* (a titer of ⩾32 and ⩾128, respectively). In these 4 patients, the diagnoses were further confirmed by detection of rickettsial DNA in skin biopsy samples by PCR, while culture for *Rickettsia* was negative. Forty-six patients were diagnosed by seroconversion observed when serum samples obtained during acute infection were compared to those obtained during convalescence, which is considered as a strong evidence of recent infection. No significant level of IgM or IgG antibodies against *R. conorii* was detected in patients with fatal cases of MSF.

The patient populations infected with *R. conorii* ISF or Malish strains, both those with fatal infections and those with nonfatal infections, were similar with respect to age, the period of time between the onset of symptoms and first visit to the physician, and length of hospitalization (table 1). There was a delay of 1 day in receipt of treatment for patients with nonfatal Malish strain infection, compared with patients with nonfatal ISF strain infection (a median of 5 days vs. a median of 4 days). No significant difference in the incidence of underlying disease or history of animal contact was detected between patients infected with the ISF strain and patients infected with the Malish strain (table 2). Although there was no difference in the seasonal distribution of patients infected with either the ISF or Malish strain, a history of a recognized tick bite was more common in patients infected with the Malish strain than in those infected with the ISF strain (20 [63%] vs. 11 [32%]) (table 2).

Comparison of the clinical manifestations of MSF caused by the Malish strain and that caused by the ISF strain revealed tremendous overlap that would prevent clinical recognition of the strain involved. Both strains were capable of causing disease that resulted in each of the signs and symptoms, as well as the pathophysiologic conditions that caused abnormal laboratory values for several markers of organ dysfunction. Moreover, statistical analysis of the clinical signs, symptoms, and laboratory data at admission revealed few differences between patients infected with the Malish strain and those infected with the ISF strain (table 2). First, an eschar was observed in a significantly higher percentage of patients infected with the Malish strain (38 [60%]), compared with patients infected with the ISF strain (23 [38%]). However, it should be emphasized that many patients infected with the Malish strain did not have an eschar (table 2). The anatomic locations of the eschars were similar for patients with either Malish or ISF infections. An eschar was often found in the axilla and/or groin (15 [25%]), legs (15 [25%]), and trunk (12 [20%]), and only 3 patients infected with *R. conorii* Malish strain presented with more than 1 eschar. Second, ISF strain–infected patients suffered nausea and vomiting more often than Malish strain–infected patients (table 2), suggesting significant gastrointestinal involvement in patients with ISF strain infection. Third, compared with Malish strain–infected patients, a higher percentage of patients with ISF strain infection had significantly elevated serum levels of total bilirubin, γ-glutamyl transferase, and alkaline phosphatase, among other altered parameters (table 2), suggesting greater hepatic involvement.

Of the 6 children infected with *R. conorii,* 4 were infected with the Malish strain and 2 with the ISF strain (table 3). The most severely ill child was a 12-year-old infected with Malish strain who did not receive treatment until the tenth day of illness. He developed encephalitis and thrombocytopenia and had markedly elevated serum levels of hepatic transaminases.

The most important observation in this study was a statistically significantly greater severity of disease in patients infected with *R. conorii* ISF strain, compared to those infected with the Malish strain (table 4). The case fatality rate for ISF strain infection was significantly greater than that for Malish strain infection (20 [29%] vs. 9 [13%]; $P = .02$), and a greater percentage of patients with ISF strain infection required admission to the ICU, compared to those with Malish strain infection (25 [36%] vs. 15 [22%]; $P = .061$). Analysis of the relationship between comorbidities and a fatal MSF outcome demonstrated that alcoholism was a statistically significant host condition that was a risk factor for a fatal outcome (table 4).

Analysis of the clinical and laboratory data for fatal and nonfatal infections provided a picture of the pathophysiology of severe MSF in a large series of patients who had been documented specifically to have been infected with *R. conorii* by isolation of *Rickettsia* species or by molecular diagnosis and genetic identification of the agent, rather than by a diagnosis based on clinical manifestations or serologic analysis that did not distinguish among various spotted fever–group rickettsioses. Patients with fatal cases of etiologically documented *R. conorii* infection were more likely to have severe involvement of multiple organ systems as indicated by significantly greater occurrence of petechial rash, nausea, vomiting, diarrhea, prostration, obtundation and/or confusion, dehydration, tachypnea, and hepatomegaly (table 4) Also, patients with fatal infection were more likely to have leukocytosis (>11,300 cells/μL), prolonged partial thromboplastin time, and elevated serum concentrations of urea, creatinine, bilirubin, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, and creatine kinase (table 4) at the time of admission. Of the patients with fatal cases, 5 did not have rash or eschar at the time of admission. Fever and rash were present significantly more often among patients with nonfatal cases, compared with those who had fatal cases. Multivariate analysis identified 3 variables as independent predictors associated with fatal outcome: hyperbilirubinemia (OR, 25.99 [95% confidence interval {CI}, 5.90–137.93]; $P < .001$), acute renal failure (OR, 18.10 [95% CI, 1.89– 173.35]; $P = .012$), and absence of rash (OR, 0.03 [95% CI, 0.00– 1.22]; $P = .064$). The results of the goodness-of-fit test (Hosmer-Lemeshow) showed that the model behaved with good fitness ($P = .125$)

Data on antimicrobial therapy were available for 129 adult patients (64 infected with the Malish strain and 65 infected with the ISF strain). Doxycycline was the drug most often used to treat patients with MSF; it was administered to 57 patients (89%) infected with the Malish strain and to 46 (71%) infected with the ISF strain. Doxycycline was used in combination with a fluoroquinolone for 2 patients (3%) infected with the Malish strain and for 7 patients (11%) infected with the ISF strain. Six patients (9%) infected with the ISF strain received therapy with a fluoroquinolone alone. Of 64 patients infected with the Malish strain, 2 (3%) received no treatment, 2 (3%) were treated with a β-lactam drug, and 1 (2%) was treated with rifampin. Of 65 patients infected with the ISF strain, 5 (8%) patients received no treatment

Table 3. Clinical and laboratory data for 6 children with Mediterranean spotted fever.

	Patient number					
Variable	1	2	3	4	5	6
Infecting strain	ISF	ISF	Malish	Malish	Malish	Malish
Age	4 years	6 years	1 years	5 months	6 months	12 years
Treatment interval						
1st symptoms to specific therapy, days	2	4	4	NA	NA	10
Admission to discharge, days	0	9	9	9	0	11
Clinical sign or symptom						
Fever	Yes	Yes	Yes	Yes	Yes	Yes
Eschar	No	No	No	No	No	Yes
Rash	Maculopapular	Petechial	Maculopapular	Maculopapular	Maculopapular	Maculopapular
Headache	. . .	. . .	. . .	. . .	. . .	Yes
Myalgia	. . .	. . .	. . .	. . .	Yes	Yes
Gastrointestinal signs	Vomiting	. . .	. . .	. . .	Vomiting	. . .
Hepatomegaly	. . .	. . .	. . .	. . .	. . .	. . .
Splenomegaly	Yes	. . .	. . .	. . .	. . .	. . .
Complications	. . .	. . .	. . .	. . .	. . .	Encephalitis
Antimicrobial drugs received	Azithromycin	Doxycycline	Azithromycin	Chloramphenicol	None	Azithromycin
Laboratory data						
Leukocyte count, cells/μL	8920	2260	9930	17,800	. . .	13,870
Platelet count, platelets/ μL	177,000	106,000	137,000	177,000	. . .	88,000
Urea level, mg/dL	21	15	27	. . .	. . .	. . .
Creatinine level, mg/dL	0.4	0.5	0.3	0.6	. . .	. . .
Alanine transaminase level, U/L	56	246	347	. . .	. . .	826
Aspartate transaminase level, U/L	58	285	365	. . .	. . .	1117

NOTE. ISF, Israeli spotted fever.

and 1 (2%) was treated with a β-lactam drug. No statistically significant differences were found between the groups of patients with respect to therapeutic regimens ($P = .472$). Among the 7 patients who did not receive treatment, 2 survived and 5 died.

Of the 6 child patients, 3 received treatment with a macrolide (azithromycin), 1 received chloramphenicol, and 1 received doxycycline. The 6-month-old child recovered without specific treatment. No fatal cases occurred in children (table 3).

DISCUSSION

To our knowledge, this study represents the largest case series of patients with a confirmed diagnosis of MSF based on documentation of *R. conorii* infection and the largest series that includes identification of the strain, rather than only clinical diagnosis or non–species specific serologic diagnosis. Owing to the presence of shared protein and lipopolysaccharide antigens among spotted fever–group rickettsiae, it is extremely difficult to distinguish between infections due to closely related rickettsiae by serologic or immunohistochemical methods [14, 15]. In the Mediterranean basin, patients with similar clinical manifestations have been documented (by isolation of *Rickettsia* species) to be infected with *R. sibirica* mongolotimonae strain, *R. akari,* and *R. massiliae*, all of which would have produced antibodies that were cross-reactive with *R. conorii* [11, 16, 17]. Isolation and/or PCR detection followed by genetic characterization can determine the genotype of the organism to the level of genus, species, and strain. Previous studies by Sousa et al. (Portugal) and Giammanco et al. (Italy) compared some of the clinical manifestations of MSF caused by ISF and Malish strains [3, 18].

Table 4. Univariate analysis of epidemiologic, clinical, and laboratory findings in patients with fatal and nonfatal Mediterranean spotted fever.

Finding, by type	Total[a]	Type of infection, no. (%) of patients		OR adjusted by strain (95% CI)	*P*
		Nonfatal	Fatal		
Epidemiologic finding					
Rickettsial strain					
Malish	71	62 (87)	9 (13)	1	. . .
ISF	69	49 (71)	20 (29)	2.81 (1.18–6.72)	**.020**
Animal contact[b]	88	69 (90)	11 (100)	NA	NA
Tick bite	66	27 (50)	4 (33)	0.68 (0.17–2.72)	.587
Clinical finding					
Fever	137	106 (97)	23 (82)	0.11 (0.23–0.54)	**.006**
Eschar	123	53 (52)	8 (36)	0.59 (0.22–1.56)	.589
Rash					
Any	137	105 (97)	23 (79)	0.12 (0.03–0.52)	**.005**
Maculopapular		102 (97)	18 (78)	1	. . .
Petechial		3 (3)	5 (22)	10.93 (2.29–53.83)	**.003**
Asthenia	80	59 (94)	15 (88)	0.63 (0.097–4.121)	.633
Headache	77	47 (76)	13 (87)	2.34 (0.43–12.73)	.326
Myalgia	85	58 (83)	14 (93)	3.08 (0.35–27.19)	.311
Arthralgia	69	31 (55)	7 (54)	1.19 (0.33–4.23)	.792
Abdominal pain	70	11 (29)	8 (57)	4.56 (1.23–16.85)	**.023**
Anorexia	65	37 (66)	9 (82)	2.58 (0.46–14.50)	.282
Nausea	75	25 (40)	11 (92)	12.5 (1.48–106.94)	**.021**
Vomiting	84	22 (33)	14 (82)	7.5 (1.88–30.12)	**.004**
Diarrhea	79	16 (26)	12 (71)	6.38 (1.81–22.42)	**.004**
Prostration	81	36 (58)	17 (89)	6.73 (1.37–33.03)	**.019**
Obtundation	100	22 (27)	12 (67)	9.26 (2.61–32.91)	**.001**
Dehydration	72	18 (33)	13 (76)	7.55 (1.95–29.29)	**.003**
Tachypnea	63	11 (25)	17 (89)	29.17 (5.35–158.97)	**<.001**
Chest pain	53	6 (15)	3 (25)	1.79 (0.35–9.18)	.486
Hepatomegaly	60	12 (24)	6 (67)	7.37 (1.46–37.04)	**.015**
Splenomegaly	57	4 (8)	1 (17)	2.00 (0.17–23.77)	.584
Comorbidity					
Alcoholism	103	11 (13)	7 (47)	**6.994** (1.99–24.64)	**.002**
Tobacco use	87	10 (14)	3 (18)	1.286 (0.30–5.54)	.735
Diabetes	107	8 (9)	1 (6)	0.745 (0.08–6.65)	.793
Cardiac failure	105	11 (13)	3 (18)	1.773 (0.41–7.60)	.440
Respiratory failure	85	5 (7)	1 (6)	0.705 (0.07–6.79)	.763
Chronic renal failure	85	3 (4)	0	. . .	. . .
Hypertension	89	13 (18)	4 (25)	1.839 (0.48–7.07)	.375
Laboratory finding[c]					
Anemia, hemoglobin level <11 g/dL	118	18 (20)	3 (11)	0.59 (0.15–2.20)	.424
Leukopenia, <4400 cells/μL	121	13 (14)	3 (12)	1.30 (0.31–5.59)	.716
Leukocytosis, >11,300 cells/μL	121	10 (11)	12 (46)	11.13 (3.37–36.80)	**<.001**
Thrombocytopenia [d]	122	73 (78)	27 (96)	6.8 (0.86–53.78)	.069
Prothrombin time >13 s	84	34 (50)	9 (60)	2.14 (0.59–7.74)	.246
Partial thromboplastin time >35 s	95	22 (29)	17 (85)	36.78 (4.53–298.44)	**.001**
Glucose level >110 mg/dL	101	52 (68)	10 (42)	0.36 (0.14–0.95)	**.038**
Urea level[d]	112	36 (42)	25 (93)	21.02 (4.50–98.42)	**<.001**
Creatinine level, >1.2 mg/dL	120	42 (46)	26 (93)	20.81 (4.44–97.46)	**<.001**
Sodium <145 mmol/L	120	48 (52)	8 (29)	0.39 (0.15–0.99)	**.047**
Potassium <3.5 mmol/L	116	15 (16)	2 (8)	0.49 (0.10–2.36)	.375
Total bilirubin level, >1.2 mg/dL	109	16 (19)	23 (92)	45.53 (9.62–215.0)	**<.001**

(continued)

Table 4. ***(Continued)***

Finding, by type	Total[a]	Type of infection, no. (%) of patients: Nonfatal	Fatal	OR adjusted by strain (95% CI)	*P*
Aspartate transaminase level[d]	112	70 (81)	25 (96)	4.73 (0.59–38.67)	.147
γ-Glutamyl transferase level[d]	77	34 (56)	15 (94)	9.58 (1.16–78.92)	**.036**
Alkaline phosphatase level[d]	88	19 (27)	15 (88)	17.52 (3.59–85.44)	**<.001**
Creatine kinase level[d]	75	20 (35)	12 (67)	5.06 (1.50–17.09)	**.009**
C-reactive protein level, >0.5 mg/dL[b]	66	52 (98)	13 (100)	. . .	. . .

NOTE. Bold type indicates statistically significant *P* values. CI, confidence interval; ISF, Israeli spotted fever; OR, odds ratio; NA, not available.

[a] Total patients with available data.

[b] OR, CI, and *P* value could not be calculated because there were no fatal cases with normal values.

[c] Children were excluded from analyses of laboratory data.

[d] For these laboratory values, the abnormal values observed were considered outside the normal range limits (either below or above those limits) established by each different hospital laboratory.

This is the first exhaustive study reporting the differences between patients infected with *R. conorii* Malish strain and patients infected with the ISF strain, as well as the microbe-related and host-related risk factors and the clinical manifestations associated with a fatal outcome. In general, patients infected with the ISF strain and patients infected with the Malish strain did not show many differences in epidemiological, clinical, or laboratory data. The most important findings documented here are that the ISF strain is more virulent than the Malish strain in the population investigated and alcoholism is a risk factor for fatal outcome in MSF. The microbial pathogenic mechanism by which the ISF strain causes more severe illness remains to be determined. With regard to host-related risk factors, previous studies have described alcoholic patients who developed severe MSF. However, the present study is the first statistical analysis involving a representative sample that documents alcoholism as a risk factor for severity of disease [19].

Our data suggest that gastrointestinal symptoms such as nausea, vomiting, and diarrhea (table 4) are prominent manifestations in patients with fatal MSF. However, these symptoms might have been related to central nervous system involvement if intracranial pressure was increased. (i.e., among the patients with fatal cases, there were many patients with obtundation and/or confusion [12 {67%}]; rickettsial infection in severe or fatal cases may result in cerebral edema and increased intracranial pressure, which are features of rickettsial encephalitis, and nausea and vomiting could be gastrointestinal symptoms that reflect central nervous system involvement). Thus, the exact mechanism that accounts for the presence of marked gastrointestinal symptoms in patients with fatal cases is not yet clear. Furthermore, there were significantly greater gastrointestinal manifestations, such as nausea and vomiting, in patients with ISF strain infection. It is possible that infection with the virulent ISF strain causes inflammation of abdominal organs secondary to rickettsial growth in blood vessels in these tissues or systemic or local production of high levels of proinflammatory cytokines and chemokines. This conclusion is consistent with our previous study showing that patients with severe MSF had very high dermal levels of proinflammatory tumor necrosis factor–α, enzymes that mediate intracellular bacterial elimination as well as tissue injury (i.e., inducible nitric oxide synthase and indoleamine dioxygenase), and RANTES, a chemokine that stimulates the migration and accumulation of T cells at the site of infection [20].

Multiple logistic regression analysis revealed an independent significant association between the presence of hyperbilirubinemia and fatal outcome in a greater percentage of patients with ISF strain infection, compared to patients with Malish strain infection. Whether hyperbilirubinemia is related to cholestasis, hemolysis, or other mechanisms remains to be determined. Nevertheless, it is clearly evident that hepatic involvement marked by hepatomegaly and elevated serum levels of alanine transaminase, alkaline phosphatase, and γ-glutamyl transferase is strongly associated with a fatal outcome. Histological studies have shown that the hepatic pathology in patients with MSF, namely focal necrosis and mononuclear lobular and portal triad inflammation, is not associated with fatal disease [21]. The lack of correlation between pathology and fatal disease in combination with the strong association between fatal disease and abnormal values for several laboratory markers of organ dysfunction confirm the clinical diagnosis of multiple organ system involvement as an important factor in fatal rickettsial diseases. Indeed, there was significant evidence of greater injury in numerous organ systems for patients with fatal cases, compared with those with nonfatal cases, including injury to vascular endothelium in the brain (obtundation and/or confusion), lungs (tachypnea), the coagulation system (prolonged partial thromboplastin time), and skin (petechial rash). Systemic hypotension with decreased renal perfusion and glomerular filtration rate are also most likely the pathophysiologic mechanism behind the greater incidence of acute renal failure in patients with fatal infections [22].

This study reveals a lower incidence of eschars in patients with MSF due to the ISF strain, compared with patients infected with

the Malish strain. The significant difference in eschar formation between MSF due to the ISF strain and that due to the Malish strain was not detected in our previous study, which we attribute to the nonrepresentative number of patients involved in our previous analysis. Consistent with this conclusion is the finding that many patients infected with the Malish strain in this study also did not have an eschar, as described previously. Furthermore, the current study includes a larger number of patients with fatal infection who were infected with either the Malish or the ISF strain, and some of them did not have eschars. Similarly, the case fatality rate for ISF strain infection in this study was significantly greater than that for Malish strain infection (29% vs. 13%; $P = .02$), and a greater percentage of ISF strain–infected patients required admission to the ICU, compared with Malish strain–infected patients (36% vs. 22%; $P = .061$). It is not yet clear whether the lower incidence of eschar in patients with fatal ISF strain infection is a prognostic factor that indicates an ineffective or detrimental host immune response against *Rickettsia* or a confounding factor that causes delayed diagnosis of these patients and thus disease progression. We favor the first possibility because our data show that the ISF strain–infected patients received antirickettsial treatment earlier in the course of disease than the Malish strain–infected patients.

The substantial case fatality rate for MSF in Portugal differs remarkably from that reported in some other regions of the Mediterranean area, and there are notable differences between Portugal's rate and the rates of countries that are near Portugal, such as France and Spain. The possibility that some *Rickettsia* strains are more virulent than others is a reasonable hypothesis to examine in such areas. It is conceivable that another, less virulent human pathogen, such as *R. massiliae* or *R. monacensis,* might be the etiologic agent of spotted fever rickettsiosis in some geographic locations, such as in the northeast of Spain [23, 24].

The reason for the more frequent occurrence of a history of tick bite in patients infected with the Malish strain is not evident. Although different vector species or tick stages could be hypothesized to have transmitted the infection without detection, most of the cases occurred in similar months for both strains, and *Rhipicephalus sanguineus* is the only recognized tick host of *R. conorii* in Portugal [25].

The lower incidence of rash and eschar as well as *Rickettsia*-specific IgM or IgG antibodies in patients with fatal cases emphasizes the need to consider the diagnosis of MSF for patients with unexplained febrile illness, even in the absence of these signs and serological markers, in regions where MSF is endemic. This study reveals that diagnosis by PCR amplification of rickettsial DNA from skin biopsy samples is a useful method for establishing the etiologic agent. PCR was the only method by which the diagnosis was established in 47 patients. Additionally, 8 patients were diagnosed by both PCR and blood culture. Of 109 skin biopsy samples examined by PCR in this study, 55 (50%) samples were positive and 54 (50%) were negative for rickettsial DNA. Among the patients with skin biopsy samples that were PCR-negative for rickettsial DNA, 22 patients had rickettsial infection confirmed by serologic analysis.

Further analysis of the genotype of the *Rickettsia* allows identification of the agent to the strain level with the potential for investigation of differences in specific hypothetical virulence factors including adhesins (outer membrane proteins A and B) [26–28], actin mobility (RickA) [29], and membranolytic enzymes (phospholipase D and hemolysins A and C) [30–32]. Advances in diagnosis and knowledge of the pathogenic mechanisms of rickettsioses require further attention.

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